Supporting Information

Reduction- and Thermo-Sensitive Star Polypeptide Micelles and Hydrogels for On-Demand Drug Delivery

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Materials. Diethylene glycol monomethyl ether (EG_2, C.P. grade), Ethylenediamine (A.R. grade), Methyl acrylate (MA, A.R. grade) and tetrahydrofuran (THF, A.R. grade) were purchased from Shanghai Sinopharm Chemical Reagent Corporation and distilled before use. N,N-Dimethylformamide (DMF, A.R. grade) was distilled from calcium hydride under reduced pressure and stored over 4 Å molecular sieves. 1,6-Diphenyl-1,3,5-hexatriene (DPH, Aldrich), L-glutamic acid (≥98%, GL Biochem Shanghai Ltd), Cystamine dihydrochloride (98%, Aldrich), and triphosgene (99%, Aladdin Chemistry Ltd) were used as received.

Methods. Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer at frequencies ranging from 400 to 4000 cm^{-1}. Samples were thoroughly mixed with KBr and pressed into pellet form. ^1H NMR spectrum was performed at room temperature on a Varian Mercury-400 spectrometer. Molecular weight distribution (M_w/M_n) of polymer was determined on a gel permeation chromatograph (GPC, HLC-8320, Tosoh Corporation) equipped with a

refractive index detector at 30 °C. The elution phase was DMF (0.01 mol.L^{-1} LiBr, elution rate: 0.6 mL/min), and polymethylmethacrylate was used as the calibration standard. The mean size of nanoparticles was determined by dynamic light scattering (DLS) using a Malvern Nano_S instrument (Malvern, UK). The solution of nanoparticles was performed at a scattering angle of 90 ^{o}C and at 25 ^{o}C. All the measurements were repeated three times, and the average values reported are the mean diameter ± standard deviation. UV-vis spectra of samples were recorded at room temperature using a Spectrumlab54 UV-visible spectrophotometer. Transmission electron microscopy (TEM) was performed without negative staining using a JEM-2010/INCA OXFORD TEM (JEOL/OXFORD) at a 200 kV accelerating voltage. Samples were deposited onto the surface of 300 mesh Formvar-carbon film-coated copper grids, and excess solution was quickly wicked away with a filter paper. The rheological behavior of the hydrogels was investigated by a TA-ARG2 rheometer using 40 mm parallel-plate geometry at 25 °C. The gap distance between the two plates was fixed at 0.3 mm.

Preparation of the Disulfide-Bond-Cored Tetra(amine). The disulfide-bond-cored tetra(amine), as light yellow viscous oil, was synthesized according to the previous publication.[1] ^{1}H NMR (400MHz DMSO-d6): δ (ppm): 2.08-2.25 (t, 8H, NCH_2CH_2CONH), 2.50-2.60 (t, 8H, $CONHCH_2CH_2NH_2$), 2.60-2.70 (t, 12H, SCH_2CH_2N (CH_2) CH_2CH2), 2.71-2.80(t, 4H, SCH2CH2N), 2.95-3.05(m, 8H, $CONHCH_2CH_2NH_2$). FT-IR (KBr, cm-1): 3260, 3075, 2925, 2855, 1660, 1555, 1385, 1240, 1120.

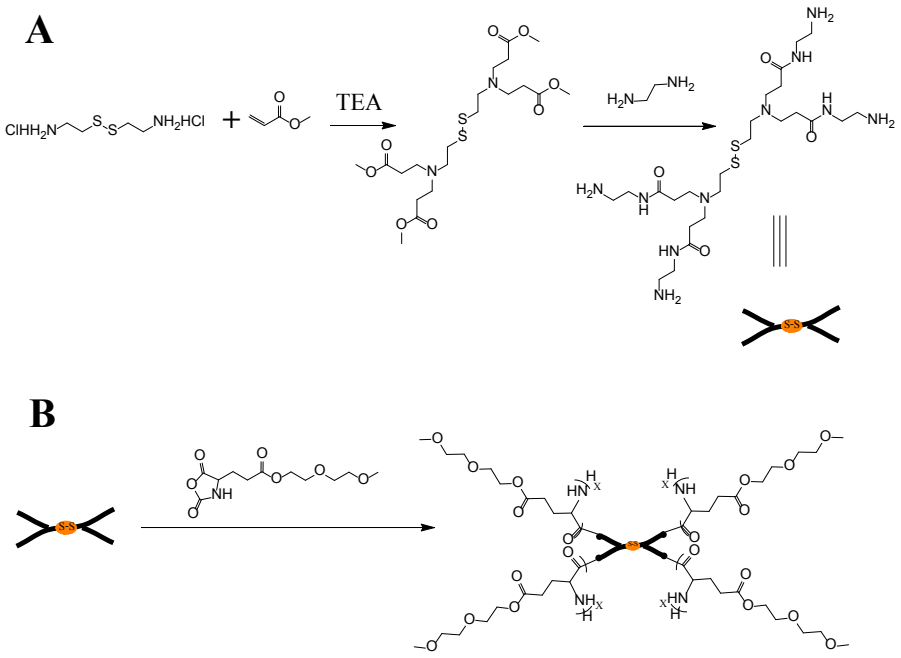
Synthesis of EG_2-Glu-NCA. EG_2-L-Glutamate N-carboxyanhydride (EG_2-Glu-NCA) monomer, as a colorless viscous oil, was synthesized according to our previous publication.[2] ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm): 2.06-2.40 (m, 2H, NHCHCH_2CH_2), 2.45-2.62 (m, 2H, CH_2CH_2COO), 3.34-3.40 (s, 3H, CH_3O), 3.54-3.59 (m, 2H, CH_2CH_2O), 3.65-3.78 (m, 4H, OCH_2CH_2OCH_3), 4.22-4.38 (m,2H, COOCH_2CH_2), 4.39-4.42 (m, 1H, CONHC*H*CH_2), 7.12-7.18 (s, 1H, CON*H*CH). FT-IR (KBr, cm^{-1}): 3292, 2925, 1852, 1785, 1735, 1652, 1547, 1106, 916. TOF-MS (m/z) $[M + H]^+$: calcd for $C_{11}H_{17}NO_7$, 276.256; found, 276.080.

Preparation of Star Polypeptides. A series of Poly-L-EG_2-Glu star polypeptides was synthesized from the ROP of EG_2-Glu-NCA using Tetra (amine) as initiator in DMF solution at 30 ^{o}C. As a typical example, EG_2-Glu-NCA (275.0 mg, 1.00 mmol) was dissolved in dry DMF (1 mg/mL), and Tetra (amine) (0.013 mmol) in DMF solution was added to the stirred solution under N_2 atmosphere. After stirred vigorously at 30 ^{o}C for 72 h, the solvent was partially removed on a rotary evaporator. The solution was diluted with THF (3mL) and precipitated dropwise into a large excess of diethyl ether (30mL), and then the white product was washed twice with diethyl ether (10mL). The resulting precipitate was filtered and dried in vacuo to give a white solid product (170.4 mg, 70.3 % yield). ^{1}H NMR (400 MHz, DMSO-d6): δ (ppm): 1.66-2.10 (br, 136H, NHCHCH_2CH_2), 2.15-2.22 (br, 8H,NCH_2CH_2CONH), 2.25-2.50 (br, 136H, CH_2CH_2COO), 2.60-2.70 (br, 12H, SCH_2CH_2N(CH_2)CH_2CH_2), 2.73-2.79 (br, 4H, SCH_2CH_2), 3.05-3.15 (br, 8H, NHCH_2CH_2), 3.18-3.25 (s, 204H, CH_3O), 3.38-3.45 (br, 136H, CH_2CH_2OCH_3), 3.46-3.65 (br, 272H, CH_2OCH_2CH_2OCH_3), 3.90-4.30 (br,

204H, COOCH_2CH$_2$, CONHCHCH$_2$),. FT-IR (KBr, cm^{-1}): 3292, 2910, 1735, 1655, 1551, 1115. $M_{n,\,GPC}$ = 15540 , M_w/M_n = 1.64.

Self-Assembled Polypeptide Micelles in Aqueous Solution. The self-assembled polypeptide micelles can be obtained by dissolution in water or prepared by a widely-used dialysis method, the details can be referred to our previous publications.[2] In order to obtain the uniform micelles, the dialysis method was used in this work. The obtained micelles solution with a concentration of 0.5 mg/mL was stored at room temperature before measurement, and both the mean size and morphology of micelles were determined by DLS and TEM, respectively.

Preparation of Star Polypeptide Hydrogels. S1 (60 mg) was dissolved in 1.5 mL of distilled water under a vigorous stirring, and then formed hydrogels in tube at room temperature. The critical gelation concentration of homopolypeptides was determined by the test-tube inversion method.



Scheme S1. Synthesis of a disulfide-bond-cored tetra(amine) by Michael-addition reaction (A); and Synthesis of star polypeptides by ring-opening polymerization of EG_2-Glu-NCA (B).

Table S1. Synthesis of both star and linear disulfide-bond-cored polypeptides.

Entry	[M]/[I][c] (mol:mol)	DMF (mL)	Yield (%)	$M_{n,NMR}$[d]	$M_{n,GPC}$[e]	M_w/M_n[e]
L1[a]	20:1	3.5	77.8	7180	7460	1.67
S1[b]	20:1	3	70.3	16510	15540	1.64
S2	40:1	3	80.1	34550	33820	1.57

a: L denotes linear; b: S denotes star-shaped; c: M = monomer, I = initiator; d: $M_{n,NMR}$ was the polymer molecular weight determined by ^{1}H NMR; e: $M_{n,GPC}$ and M_w/M_n were determined by GPC, respectively.

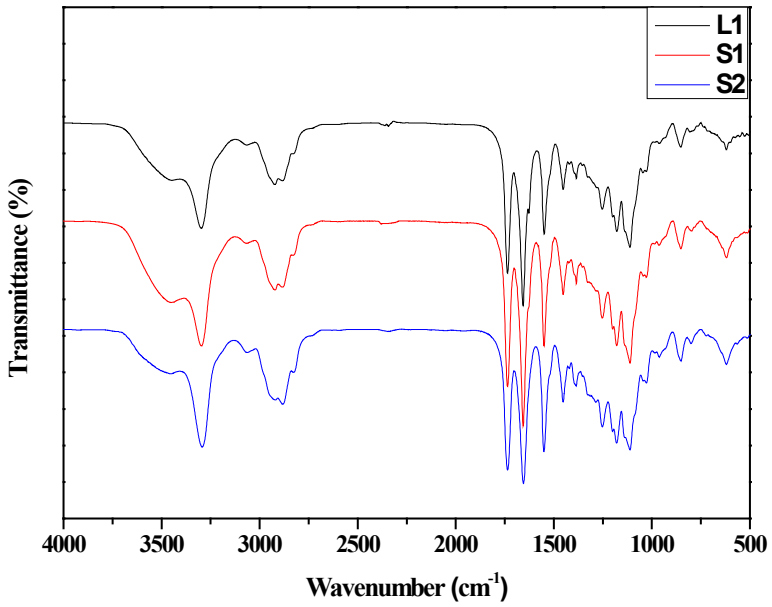


Figure S1. FT-IR spectra of polypeptides.

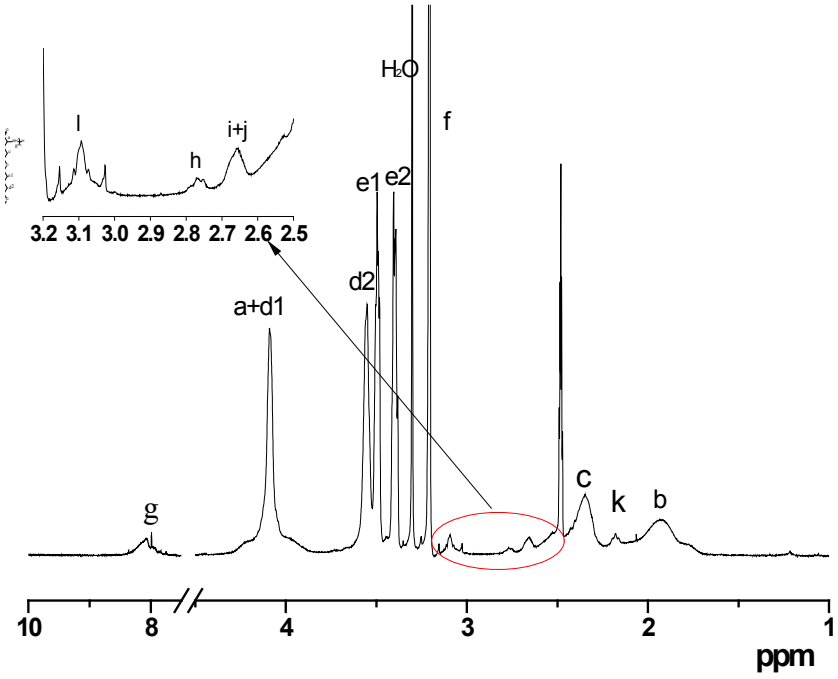


Figure S2. ^{1}H NMR of S1 in DMSO-d_6.

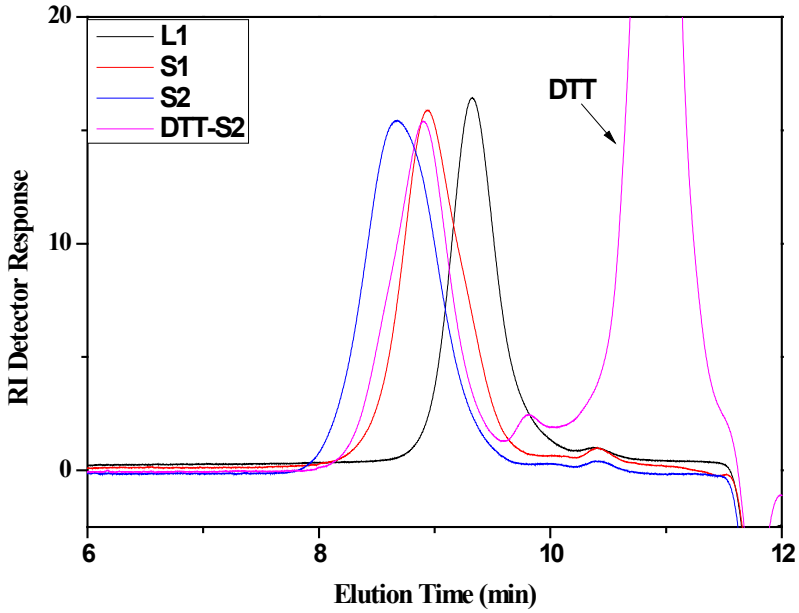


Figure S3. GPC traces of polypeptides and the DTT-reduced S2.

Note: the DTT-reduced S2 trace clearly confirmed that it was cleaved and reduced into linear subunits after addition of DTT.

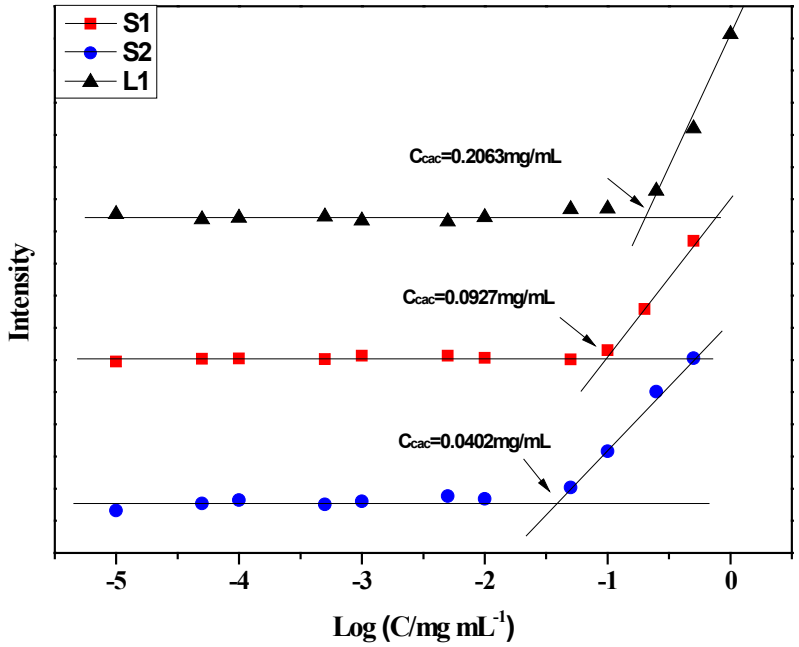


Figure S4. Relationship of the absorbance intensity of DPH as a function of the polypeptide concentration at room temperature.

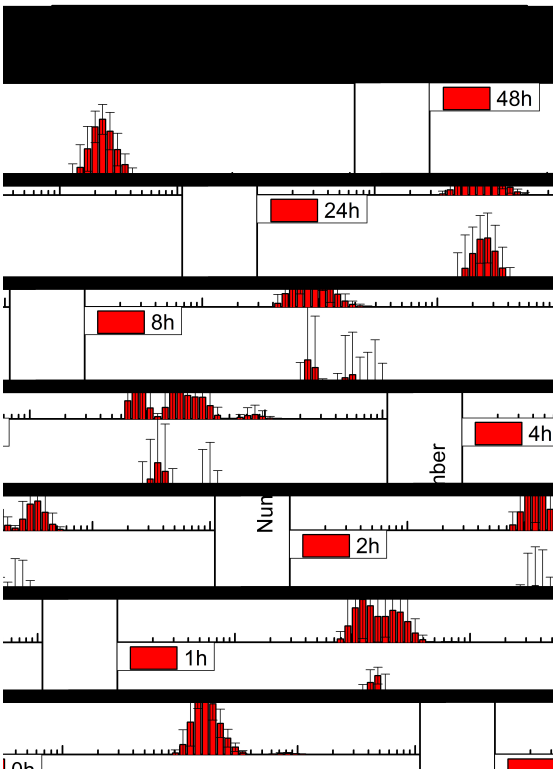


Figure S5 The number-averaged size dependence of the L1 micelles on the incubation time in the presence of 10 mM DTT at 37°C.

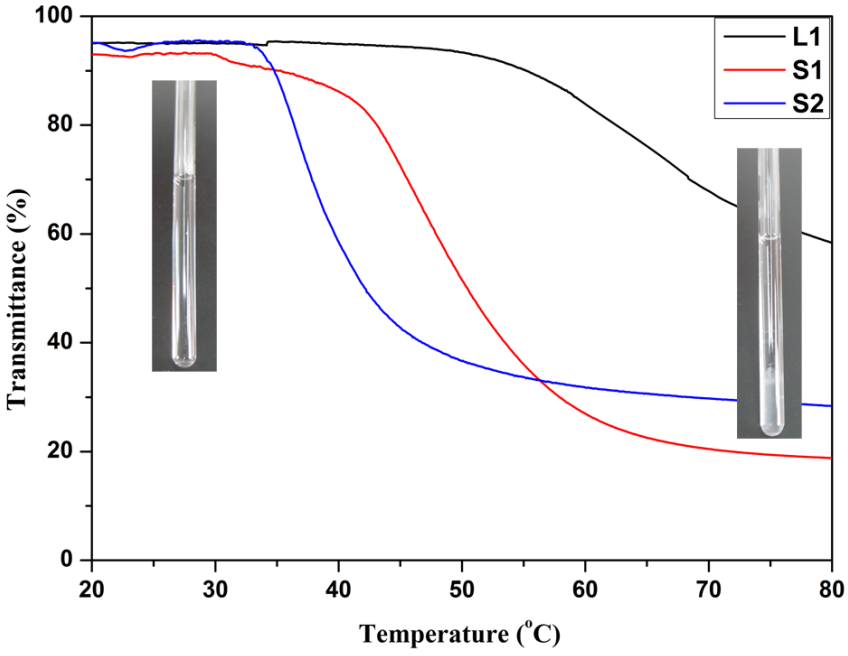


Figure S6. The transmittance dependence of the micelles solution on temperature, and inset are the digital photos.

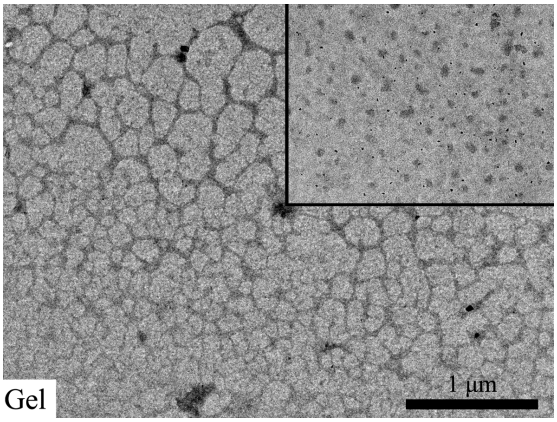


Figure S7. TEM photograph of the S1 hydrogels, and inset is the expanded region of the hydrogel microdomain.

References and Notes

1. D. A. Tomalia, B. Huang, D. R. Swanson, H. M. Brothers, II and J. W. Klimash, *Tetrahedron*, 2003, **59**, 3799-3813.

2. Y. Liao and C. M. Dong, *J. Polym. Sci. Polym. Chem.*, 2012, **50**, 1834-1843.